The Effect on Apheresis Platelet Quality During Shipment With Continued Interruption of Agitation for 24 and 48 Hours

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ABSTRACT

BACKGROUND: The role of the Armed Services Blood Program Office (ASBPO) is to ensure quality blood products are provided during peacetime and war. Delays occurring during shipment of products into the area of operation can have negative consequences on platelet concentrates (PCs), which is why liquid platelets are not being shipped and utilized in current operational theaters. In order for this to change, shipping methods that ensure functionality and viability of PCs are needed to reduce the loss of product once it arrives at its destination.

STUDY DESIGN AND METHODS: Twelve units of apheresis PLTs were split into two aliquots stored at 20-24 °C with continuous agitation, shipped overnight or 2-day air, and placed back on the rotator for the remainder of the shelf-life. Control aliquots were stored at 20-24 °C with continuous agitation. The following biochemical and pathologic markers were tested on Day 1, after return from shipment, and Day 5: pH, ATP, glucose, lactate, hypotonic shock response (HSR), extent of shape change (ESC), PLT counts, PLT swirling, flow cytometry, PO_2 and PCO_2.

RESULTS: pH levels significantly ($P<0.05$) dropped after shipment, and lactate increased ($P<0.05$) during storage; however there was no correlation of lactate accumulation to pH levels. Lactate did show a predictable relationship with glucose levels; as lactate increased, glucose decreased ($P<0.05$). PCO_2 levels increased ($P<0.01$) during transportation, however returned to baseline by Day 5. Similarly, PO_2 levels decreased ($P<0.01$, 24 hour; $P<0.05$, 48 hour) during shipment, but returned to baseline levels by expiration date. PLT morphology markers (PLT swirling and PLT activation), ATP, and PLT counts were minimally influenced ($P>0.05$) throughout the study. HSR and ESC showed no evidence of PLT damage. The Golden Hour

Platelet container maintained PCs temperature between 20 – 24 °C for over 48 hours at varying external temperature ranges during transportation.

CONCLUSION: Interruption of agitation for 24 or 48 hours during shipment produces no PLT damage measurable by these *in vitro* techniques. Although there was small reduction in pH levels, lactic acid accumulation was minimal, indicating that the PCs underwent some form of mixing during transportation, maintaining an oxidative metabolism.

Key Words: Agitation, apheresis, lactate, pH, platelet, quality

INTRODUCTION

The Armed Services Blood Program Office's (ASBPO) mission is to provide quality blood products, blood substitutes, and services for all worldwide customers in peace and war. Complicating this mission is the extended delays that can occur during shipment of blood products into areas of operation during times of conflict, making it difficult to ship platelet concentrates (PCs) and ensure that upon arrival at their destination, the product would maintain viability and functionality. The Code of Federal Regulations (CFR) states "If stored at 20 to 24 °C, a continuous gentle agitation of the platelet concentrate shall be maintained throughout the storage period."[1] However, the CFR offers no guidelines concerning shipment of PCs between periods of storage, further complicating the ASBPO's mission. In addition to the CFR, the AABB Standards[2] only state that the total duration of interruption of agitation should not exceed 24 hours.

Several investigators have studied the effects of shipping conditions on PCs without agitation in detail. Koerner[3] showed that PCs could be well preserved under shipping conditions for up to 10 hours. Moroff & George[4] maintained PCs without agitation for 24 hours to simulate worst-case shipping conditions. PCs were stored in a standard American Red Cross card-board blood shipment box (9½" x 11½" x 11½" with 1-inch styrofoam lining). There was a significant ($P<0.05$) decrease in pH levels, 7.27 (continuous agitation) and 7.10 (discontinuous agitation). However, the results from discharge of lactate dehydrogenase (LDH) from platelet cytoplasm expressed as percent total platelet LDH, 8.6 (continuous agitation) and 6.6 (discontinuous agitation), favored discontinuous agitation.

Hunter et al.[5] interrupted agitation for periods of 1, 2, and 3 days by either stopping the agitator or storing PCs in a shipping container (17½" x 15" x 15" card-board box with 3-inch

styrofoam) that remained stationary (simulated shipping conditions). After the interruption of agitation period was complete, PCs were placed back on the agitator for the remainder of the storage period (5 days total). Lactic acid production and PO_2 increased during interruption, but returned to baseline levels when agitation was resumed after a 24-hour interruption. They demonstrated that a 24-hour interruption of agitation, on the agitator or within a shipping container, does not appear to injure platelets, as long as PCs resume some form of agitation. Interruption for periods of 2 and 3 days however, resulted in increased lactic acid production capable of lowering pH levels to ≤6.5. They noted this drop in pH occurred when platelet counts exceeded 2.5×10^{12}/L and 1.5×10^{12}/L (equivalent to 1.25×10^{11} and 0.75×10^{11}).

Simon & Sierra[6] provided evidence that PCs could be transported up to 12 hours, 6 hours without strict temperature control. Platelet viability (survival in days) and pH levels were assessed on four groups of PCs: control group stored for 72 hours with continuous agitation (9.2 days, pH 6.7); Group I stored for 72 hours, transported in an automobile driven intermittently for 4 hours (9.6 days, pH 6.7); Group II stored for 48 hours, transported for 4 hours (8.8 days, pH 6.8); Group III stored for 24 hours, transported for 12 hours, and then stored on an agitator for 36 hours (8.8 days, pH 6.6). They recommended that PCs be shipped early in their shelf-life, 48 hours or less with a platelet count $<1.4 \times 10^6/\mu L$ and plasma volume of 50 – 70 mL.

Thus, we believe that the effect of shipping conditions on PCs without continuous agitation has been incompletely studied. Most PC studies have been performed with simulated shipping conditions only,[3-5] storing PCs in a stationary shipping container, yielding results that are not a true representation of the shipping environment of blood products. We feel that during normal transportation of liquid blood products, PCs in particular, that the shipping container will undergo a certain degree of movement, providing PCs with adequate agitation in order to

maintain active oxidative phosphorylation and a pH ≥6.7. Furthermore, most investigations into the adverse effects on PCs during interruption of agitation have been assessed on PCs prepared from random donors, not PCs from plateletpheresis. PCs that are collected by apheresis technique are of interest to the industry due to the growing trend of apheresis collection for platelet products. Therefore, the affects of shipping on the quality of apheresis PCs needs to be assessed.[5]

The present study was undertaken to determine the effect on apheresis platelet quality during shipment with interruption of continuous agitation for 24 and 48 hours. We measured conventional metabolic values [lactate accumulation, glucose, pH levels, hypotonic shock response (HSR), and ATP] during 5 days of storage and/or shipment and correlated them with platelet morphology [extent of shape change (ESC), platelet swirling, and flow cell cytometry], PO_2, PCO_2, and platelet counts.

MATERIALS AND METHODS

Platelet Collection and Preparation of PCs

We collected PC units from 12 healthy donors by continuous-flow centrifugation using a dual-stage channel with LRS Chamber cell separator (Cobe Spectra Apheresis System, Gambro BCT, Inc., Lakewood, CO 80215). All units were collected from human volunteers under a human use protocol reviewed and approved by the Human Use Review Committee, Walter Reed Army Institute of Research (WRAIR). ACD-A was used as anticoagulant. The processing time was on average 100 minutes, resulting in 47 to 50-mL of ACD-A per collection bag. PCs were collected from each donor in 1-Liter collection bags made from citricized PVC (Cobe Spectra Dual Needle Extended Life Platelet set with LRS Chamber, Disposables, Gambro BCT, Lakewood, CO 80215). We separated each PC into two 287-mL (SD ± 27-mL) aliquots after completed apheresis and stored them in 1-Liter platelet bags (Cobe Spectra Dual Needle Extended Life Platelet set with LRS Chamber, Disposables, Gambro BCT, Lakewood, CO 80215).

Platelet Storage and Shipment

One 287-mL (SD ± 27-mL) aliquot from each donor was agitated continuously by axial rotation on the Helmer Platelet Rotator Model PAS40 (Helmer Labs, Inc, Noblesville, IN 46060) and maintained at 22 – 24 °C in the Helmer Platelet Incubator (Helmer Labs, Inc, Noblesville, IN 46060) for the 5-day storage period. The other 287-mL (SD ± 27-mL) aliquot from each donor was also agitated continuously by axial rotation at 22 – 24 °C until shipment by Fed Ex on Day 1. 24-hour shipment of PCs consisted of same day delivery to Walter Reed Army Medical Center (WRAMC), Washington, D.C. with overnight return shipment of PCs back to WRAIR. 48-hour shipment consisted of overnight delivery to Transfusion and Donor Services, Naval

Medical Center, San Diego, CA with overnight return shipment of PCs back to WRAIR. PCs were shipped in a prototype Golden Hour Platelet container (6¼" x 5¼" x 4¼" with phase change materials, Minnesota Thermal Science, Eden Prairie, MN 55344), which was placed in the middle of a Collins box (19 x 18½ x 16½ with styrofoam, Tidewater Container Company, Suffolk, VA) with bubble wrap surrounding the Golden Hour Platelet container on all sides. Three separate 24-hour and 48-hour shipments were executed; each shipment with 2 PC units stacked horizontally one on top of the other inside the Golden Hour Platelet container. 24 hours prior to shipment, the Golden Hour Platelet container was pre-conditioned in a 22 °C environment. Temperature was monitored during shipment by dual-channel I-loggers (Escort I-Log, New Lynn, Auckland, NZ). PCs, upon their return from either 24-hour or 48-hour shipment, were maintained on the axial rotator at 22 – 24 °C for the remainder of the 5-day storage period.

Platelet Samples for Testing

We collected samples for testing from the PC units on Day 1 prior to shipment, upon return from shipment (Day 2 or Day 3 depending on duration of shipment), and Day 5 of storage. Nonagitated bags returning from shipment were mixed carefully before sampling. We reduced the PC volume, 287-mL (SD ± 27-mL) aliquots after preparation, to an average of 256-mL (SD ± 27-mL) on Day 5.

In vitro Assays

Platelet counts were determined by the Coulter® A^C·T diff 2™ Analyzer (Coulter Corporation, A Beckman Coulter Company, Miami, FL 33196) using the Coulter method which counts and sizes cells by detecting and measuring changes in electrical resistance when a particle

in a conductive liquid passes through a small aperture. WBCs and RBCs were also counted on the Coulter® A^C·T diff 2™ Analyzer.

pH, PO_2 and PCO_2 measurements were made on the Ciba Corning 855 Blood Gas Analyzer (Bayer Corporation, Tarrytown, NY 10591) with a Co-oximetry module using ion selective electrodes.

HSR and ESC were determined as previously described[7] on the SPA-2000 (Chrono-Log Corporation, Havertown, PA 19083).

ATP levels were determined in deproteinized samples by a bioluminescent technique (Roche ATP Bioluminescence Assay Kit, HS II, Roche Diagnostics Corporation, Indianapolis, IN 46250) using a luminometer/fluorometer with plate reader and injectors (Thermo Lab Systems Fluoroskan Ascent FL, Helsinki, Finland).

Lactate levels were measured in deproteinized samples at 340 nm on the ACE® Clinical Chemistry System (AIFA Wasserman Diagnostic Technologies, LLC, West Caldwell, NJ 07006) by an enzymatic reaction that converts lactate to pyruvate.

Glucose levels were also measured in deproteinized samples at 340 nm/378 nm on the ACE® Clinical Chemistry System (AIFA Wasserman Diagnostic Technologies, LLC, West Caldwell, NJ 07006) by an enzymatic method in which hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate.

Flow cytometric analysis was carried out using the FACSort flow cytometer (Becton Dickinson, San Jose, CA 95131). PLTs were distinguished from other blood cells on the basis of their forward and side light scatter profile by measuring CD41a and CD42b (GpIIb/IIIa, GpIba), which are strongly expressed on all PLTs at all times. The data for GpIIb/III and GpIba were expressed as the percentage of fluorescence-positive gated PLTs. CD62P (P Selectin) was also

measured, which appear in significant levels upon PLT activation. The data for P Selectin were expressed as the percentage of fluorescence-positive gated PLTs.

On Day 5 of storage, swirling of the platelet suspension was scored on a 0 to 3 scale as described previously.[8]

Statistical Analysis

All results are expressed as the mean (n = 6: 24- and 48-hour shipment) ±1 SD. Significance was determined as $P<0.05$ for the hypothesized means between groups. Statistics were determined by the Student's two-tailed paired *t*-test.

RESULTS

The *in vitro* properties of platelets were retained during a 5 day storage period in which continuous agitation of PCs was discontinued during shipment for periods of 24 and 48 hours (Tables 1 and 2). The effects of shipping PCs without continuous agitation at 20 to 24°C for 24 hours are shown by the data summarized in Table 1. The effects of shipping PCs without continuous agitation at 20 to 24 °C for 48 hours are shown by the data summarized in Table 2.

In both series of the experiment, there was a significant but small decrease ($P<0.01$) in pH levels on the day of return shipment in PCs that were shipped for periods of 24 and 48 hours. When the test concentrates were subjected initially to shipment during the period of discontinuous agitation, the mean pH level was only 0.25 (24 hour shipment) and 0.20 (48 hour shipment) units less than that determined for the control concentrates, and in no case was the pH of any concentrate less than 7.0 after 5 days of storage. Lactate concentrations increased after 5 days of storage in all PCs. There was a significant rise ($P<0.05$) in lactate concentration in PC units that were subjected to discontinuous agitation for 24 and 48 hours, both on the day PCs returned from shipment, and on Day 5. However, there was no predictable relationship between lactate concentration and pH levels (Figures 1 and 2).

PCO_2 levels in PCs subjected to discontinuous agitation had a significant increase ($P<0.01$) after their shipment period for both series of the experiment. However by day 5 of storage, PCO_2 mean values fell to only 1.0 Torr (24 hour shipment) less than and 0.1 Torr (48 hour shipment) more than that which was measured for the control PC units, after being placed back on the axial rotator for the remainder of their shelf life. PO_2 levels in PCs subjected to discontinuous agitation had a significant decrease ($P<0.01$, 24 hour; $P<0.05$, 48 hour) after their shipment period for both series of the experiment. After being placed back on the axial rotator

for the remainder of the PCs shelf life, PO_2 mean levels significantly rose ($P<0.05$) to only 3.2 (24 hour shipment) and 4.9 (48 hour shipment) Torr less than that determined for the control PC units.

Differences between control and test PCs in terms of PLT counts, PLT swirling and PLT activation, were not significant for both series of the experiment. PLT counts slightly decreased, but not significantly, during the 5 day storage period. Day 5 mean counts were only 0.32 and 0.34×10^{11} (continuous and discontinuous agitation, respectively) less than Day 1 mean counts for the 24 hour shipment arm of this study. The 48 hour shipment Day 5 mean counts were 0.31 and 0.36×10^{11} (continuous and discontinuous respectively) less than the Day 1 mean counts. There was no predictable relationship between PLT counts and pH levels (Figure 3). However, there was a relationship between PLT counts and lactate concentrations (Figure 4); as lactate concentrations increased, PLT counts decreased. Swirling assessments on Day 5 correlated well with the pH levels. Of 24 PCs, all scored as 2 or 3, and all had a pH $\geq$ 7.1. The PLT surface expression of P Selectin is an indicator of PLT activation. This parameter was not influenced by the experimental conditions that the PCs were subjected to.

The data in Tables 1 and 2 show that there was no significant differences in HSR PLT quality among control units and PCs that were subjected to discontinuous agitation for periods of 24 and 48 hours. Nonetheless, HSR results, as a marker of metabolic activity expressed in percent, favors discontinuous agitation. ESC, as a marker of PLT morphology, showed a significant difference ($P<0.05$) on Day 5 among control units and PCs exposed to discontinuous agitation during shipment. ESC results also favor discontinuous agitation.

Glucose levels decreased after 5 days of storage in all PCs. There was a significant drop ($P<0.05$) in the glucose concentration in PC units that were subjected to discontinuous agitation

for 24 and 48 hours, on the day PCs returned from shipment (Days 2 and 3). Figure 5 shows the relationship between glucose and lactate levels; as glucose levels decreased, lactate increased for both series of the experiment. ATP production, however, was not significantly influenced by the experimental environment that the PCs were exposed to.

Figure 6 illustrates the typical performance of the Golden Hour Platelet container when subjected to the shipment conditions of this study. Each line represents a single evaluation for each temperature measurement inside the Golden Hour Platelet container, inside the Collins box, and external temperature. Two other independent shipment evaluations were performed for each arm of the study and resulted in similar outcomes to those shown in Figure 6.

DISCUSSION

PCs are currently stored at 22 to 24 °C for up to 5 days with continuous agitation.[1,2] Agitation is considered necessary with the beneficial effect of facilitating diffusion of gases through the walls of the containers.[9] This phenomenon is associated with the maintenance of satisfactory pH levels and post transfusion platelet viability properties[10,11] by facilitating oxygen influx and carbon dioxide efflux through the walls of the PC containers.[9] Holding or shipping platelets for extended periods in the absence of continuous agitation could have deleterious effects resulting from changes in the pH level, lactate levels, or other factors. This study was conducted to examine how platelets are affected by being transported without continuous agitation for 24 and 48 hours while temperature was maintained at 20 – 24 °C inside a new prototype shipping container (the Golden Hour Platelet container).

The changes observed in PCs shipped for 24 and 48 hours without continuous agitation were minimal. The reduction in the pH levels, albeit small, probably were due to the slightly decreased transmission of oxygen and carbon dioxide across the plastic container walls during the shipment period. Under no test conditions did discontinuous agitation affect a change great enough to result in any value outside the range expected for stored PCs on the basis of previous studies.[12,13]

The results from this study confirm and extend the results of Simon and Sierra,[6] who showed that PCs could be transported to distant locations early in the PC's shelf life. We found that an interruption of continuous agitation for a 1 or 2 day period did not excessively accelerate the production of lactic acid, so that it did not result in a drop of pH levels to less than 7.0 on Day 5 of storage; contradicting the data presented by Hunter et al.[5] They concluded that the increase in production of lactic acid occurs while agitation is interrupted. When agitation was

resumed, the rate of production of lactic acid returned to baseline. We believe that the accumulation of lactate is a continuing process, an end product of glycolysis which is one of the metabolic pathways in platelets. Our data supports Kilkson et al.[14] findings that lactic acid production will occur during the storage period, even in the presence of adequate O_2. As the data in Figure 5 indicates, glucose levels dropped as lactate increased. However, lactate levels did not exceed 20 mmol/L, so that bicarbonate was not completely utilized buffering the other end product of glycolysis, hydrogen ions, thus avoiding the rapid decrease in pH levels.[15]

The periodic mixing that the shipping box underwent during transportation supplied adequate influx of O_2 and efflux of CO_2 through the PC container wall, even though PCO_2 levels in PCs subjected to discontinuous agitation had a significant increase ($P<0.01$) and PO_2 levels had a significant decrease ($P<0.01$, 24 hour; $P<0.05$, 48 hour) after their shipment period for both series of the experiments, reinforcing the results of Mitchell et al.[16] We believe this phenomenon may be due, in part, to PC units being stacked horizontally one on top of the other inside the Golden Hour Platelet container (2 units to a container), not allowing maximum gas exchange across the container walls with the surrounding air, as prescribed by Holme et al.[17] One encouraging aspect of this study was that the morphology scoring property was not influenced by discontinuous agitation for the duration of shipment, since the retention of morphologic characteristics during storage has been associated with satisfactory post-transfusion viability properties.[18]

Hunter et al.[5] also suggested that simply stopping agitation does eventually damage PLTs, with all of the damage related to an acceleration of the production of lactic acid, the extent of which correlates with the total duration of the interruption of agitation. Accelerated production of lactic acid does not damage the platelets unless the pH drops to or below 6.5. Therefore,

interruption of continuous agitation under the circumstances of shipment is not damaging. However, we must add two caveats to that statement. In general blood banking processes, agitation might be interrupted from time of collection to time of transfusion often with varying lengths of interruption, where damage to PCs might be a cumulative affect, and temperature was maintained at 22 to 24 °C during shipment of the PCs. Our study did not look at the effects of discontinuous agitation after the PCs returned from shipment, as PCs were maintained on the axial rotator for the remainder of the storage period. Nor did we study the effects on PLT quality without strict temperature control, as PCs were transported using a new shipping container in tandem with a new shipping protocol (Golden Hour Platelet container placed inside a Collins box with appropriate packing materials).

Blood and blood products for the ASBPO's mission are collected and processed at supporting bases; Joint Services blood donor centers known as Armed Services Blood Bank Centers (ASBBCs), U.S. Army Blood Donor Centers, U.S. Air Force Blood Donor Centers, and U.S. Navy Blood Donor Centers send blood collected at their sites to the Armed Services Whole Blood Processing Laboratories (ASWBPLs). When necessary, blood may be acquired from civilian blood donor centers. This blood is also sent to the ASWBPLs. The ASWBPL sends blood into the theater of operations by two methods: 1. pre-positioning some frozen blood at Blood Product Depots (BPDs), or 2. sending blood and blood components to Blood Transshipment Centers (BTCs) or Transportable Blood Transshipment Centers (TBTCs), which then forward the blood products to the Blood Supply Units (BSUs). The BTCs, TBTCs, BPDs and BSUs all are capable of sending blood to the following groups that transfuse blood to those injured in theater: forward surgery units, theater hospitals, en-route care, U.S. Navy ships, force service support groups, and allied/coalition hospitals.

Typically, 4 to 6 hours is the amount of time required to reach distances of 200 to 300 miles by ground transportation, supporting the common practice, as approved by AABB standards,[2] of shipping PCs from a blood center to a distant hospital or another destination as long as the time of transportation is less than 24 hours. However, in and around the area of conflict during a military operation, total transportation time may be delayed longer than one day. Our conclusion from this study is that *in vitro* platelet properties are not adversely affected in a biologically significant manner when PCs are shipped for 24 hours in a container capable of sustaining its load within the required temperature range. Similarly, the data indicates that platelet properties are retained when PCs are shipped for 48 hours. These results provide hospitals or surgery units at some distance from a BTC, TBTC, BPD, or BSU the advantage of receiving PCs that they can use for transfusion despite several hours of transportation.

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ABBREVIATIONS

ASBPO = Armed Services Blood Program Office; PC = platelet concentrate; CFR = Code of Federal Regulations; LDH = lactate dehydrogenase; HSR = hypotonic shock response; ESC = extent of shape change; WRAIR = Walter Reed Army Institute of Research; WRAMC = Walter Reed Army Medical Center; ASBBCs = Armed Services Blood Bank Centers; ASWBPLs = Armed Services Whole Blood Processing Laboratories; BPDs = Blood Product Depots; BTCs = Blood Transshipment Centers; TBTCs = Transportable Blood Transshipment Centers; BSUs = Blood Supply Units

TABLE 1. Effects of shipping PCs* without agitation at 20 to 24 °C for 24 hours

	Day 1		End of 24-hour Shipment		End of 5-day storage	
Assay	Continuous agitation	Discontinuous agitation	Continuous agitation	Discontinuous agitation	Continuous agitation	Discontinuous agitation
pH	7.27 ± 0.04	7.27 ± 0.03	7.35 ± 0.04	7.10 ± 0.12†	7.32 ± 0.06	7.27 ± 0.09‡
PLT count ($\times 10^{11}$)	2.98 ± 0.64	3.00 ± 0.63	2.82 ± 0.60	2.84 ± 0.61	2.66 ± 0.53	2.66 ± 0.58
ATP ($m\mathit{M}/10^{11}$ PLT)	3.86 ± 1.36	4.15 ± 1.54	3.48 ± 1.40	3.44 ± 1.17	4.18 ± 1.36	3.88 ± 0.83
Lactate (mmol/L)	2.31 ± 0.75	2.48 ± 0.70	3.34 ± 0.64	5.67 ± 2.63‡	6.91 ± 0.98	9.05 ± 2.03†
PCO_2 (mmHg)	36.8 ± 6.94	36.7 ± 6.48	28.0 ± 4.36	45.9 ± 12.5†	24.1 ± 4.18	23.1 ± 3.51
PO_2 (mmHg)	83.4 ± 22.5	79.2 ± 21.3	84.3 ± 24.8	63.9 ± 16.6†	93.5 ± 23.6	90.3 ± 22.2‡
ESC (%)	18.2 ± 5.33	20.6 ± 6.30	22.5 ± 4.30	25.2 ± 5.36	19.9 ± 5.71	22.8 ± 4.94‡
HSR (%)	58.5 ± 10.2	60.3 ± 13.0	64.5 ± 11.1	68.7 ± 8.20	56.7 ± 11.0	60.7 ± 9.37
Glucose (mmol/L)	18.1 ± 1.52	18.6 ± 2.06	16.6 ± 2.68	15.4 ± 2.25‡	15.5 ± 1.43	14.5 ± 1.46
CD62P (% gated positive)	19.9 ± 3.56	18.7 ± 3.11	22.0 ± 5.25	20.6 ± 5.51	19.4 ± 10.5	16.9 ± 9.68
PLT swirling (scale 1-3)	NT	NT	NT	NT	2.83 ± 0.41	2.33 ± 0.52

*PCs (n = 6)
†P<0.01.
‡P>0.01, P<0.05.

TABLE 2. Effects of shipping PCs* without agitation at 20 to 24 °C for 48 hours

	Day 1		End of 48-hour Shipment		End of 5-day storage	
Assay	Continuous agitation	Discontinuous agitation	Continuous agitation	Discontinuous agitation	Continuous agitation	Discontinuous agitation
pH	7.26 ± 0.02	7.26 ± 0.02	7.34 ± 0.03	7.14 ± 0.05†	7.31 ± 0.05	7.26 ± 0.07‡
PLT count ($\times 10^{11}$)	2.78 ± 0.36	2.84 ± 0.43	2.62 ± 0.36	2.65 ± 0.35	2.47 ± 0.32	2.48 ± 0.39
ATP ($mM/10^{11}$ PLT)	3.44 ± 1.61	3.68 ± 1.43	3.46 ± 1.36	3.61 ± 1.43	3.66 ± 1.50	3.49 ± 1.60
Lactate (mmol/L)	1.82 ± 0.48	1.90 ± 0.32	4.10 ± 1.02	5.59 ± 1.79†	6.61 ± 0.73	8.06 ± 1.42‡
PCO_2 (mmHg)	36.3 ± 4.85	35.6 ± 3.50	25.0 ± 3.53	36.9 ± 3.64†	21.5 ± 3.49	21.6 ± 3.92
PO_2 (mmHg)	95.2 ± 20.6	92.2 ± 24.7	101.3 ± 16.8	83.3 ± 20.3‡	111.2 ± 14.8	106.3 ± 17.0‡
ESC (%)	17.7 ± 5.83	18.2 ± 8.66	18.1 ± 5.53	22.1 ± 4.45	17.9 ± 5.99	19.9 ± 5.01‡
HSR (%)	58.7 ± 9.27	58.9 ± 5.30	67.3 ± 11.8	68.3 ± 10.8	59.5 ± 12.0	62.2 ± 8.46
Glucose (mmol/L)	17.7 ± 1.47	18.1 ± 1.26	16.5 ± 1.77	15.7 ± 1.50‡	15.1 ± 1.60	14.4 ± 1.99
CD62P (% gated positive)	20.2 ± 3.86	17.1 ± 1.59	19.6 ± 6.16	17.8 ± 6.33	17.9 ± 12.2	15.9 ± 10.9
PLT swirling (scale 1-3)	NT	NT	NT	NT	2.83 ± 0.41	2.50 ± 0.55

*PCs (n = 6)
†P<0.01.
‡P>0.01, P<0.05.

LEGENDS FOR ILLUSTRATIONS

Fig. 1. Relationship between pH on Day 1, end of 24-hour shipment (Day 2), and Day 5 of storage and lactate concentration. There was a significant rise ($P<0.05$) in lactate concentration in PC units that were subjected to discontinuous agitation during shipment for 24 hours, both on the day PCs returned from shipment (Day 2), and on Day 5. There was a significant ($P<0.01$) decrease in pH levels on the day of return shipment (Day 2) in PCs that were shipped for 24 hours, and a significant rise ($P<0.05$) in pH levels on Day 5 within 0.05 ± 0.07 of the mean pH level for the control units.

Fig. 2. Relationship between pH on Day 1, end of 48-hour shipment (Day 3), and Day 5 of storage and lactate concentration. There was a significant rise ($P<0.01$) in lactate concentration in PC units that were subjected to discontinuous agitation during shipment for 48 hours, both on the day PCs returned from shipment (Day 3), and on Day 5. There was a significant ($p<0.01$) decrease in pH levels on the day of return shipment (Day 3) in PCs that were shipped for 48 hours, and a significant rise ($P<0.05$) in pH levels on Day 5 within 0.05 ± 0.09 of the mean pH level for the control units.

Fig. 3. Relationship between pH on Day 1, end of 24-hour shipment/Day 2 (A) and 48-hour shipment/Day3 (B), and Day 5 of storage and PLT counts. PLT counts continued to slightly drop as pH levels fluctuated down (Days 2 and 3) and then back up (Day 5) for test units; pH levels fluctuated up (Days 2 and 3) and then down (Day 5) for control units.

Fig. 4. Relationship between lactate concentrations on Day 1, end of 24-hour shipment/Day 2 (A) and 48-hour shipment/Day3 (B), and Day 5 of storage and PLT counts. Lactate concentrations increased during the 5 day storage period, significantly ($P<0.05$) for test units, and PLT counts slightly decreased for the same time-frame.

Fig. 5. Relationship between lactate concentrations on Day 1, end of 24-hour shipment/Day 2 (A) and 48-hour shipment/Day3 (B), and Day 5 of storage and glucose levels. Lactate concentrations increased during the 5 day storage period, significantly ($P<0.05$) for test units, and glucose levels decreased ($P<0.05$) for both series of the experiment upon return of the shipment.

Fig. 6. Performance charts of the Golden Hour Platelet container when subjected to 24 hour shipment (A) and 48 hour shipment (B) conditions of this study. Regardless of the temperature inside the Collins box or external temperature, PCs, inside the Golden Hour Platelet container, were maintained at 22.8°C (24 hours) and 21.3°C (48 hours).

FIGURE 1.

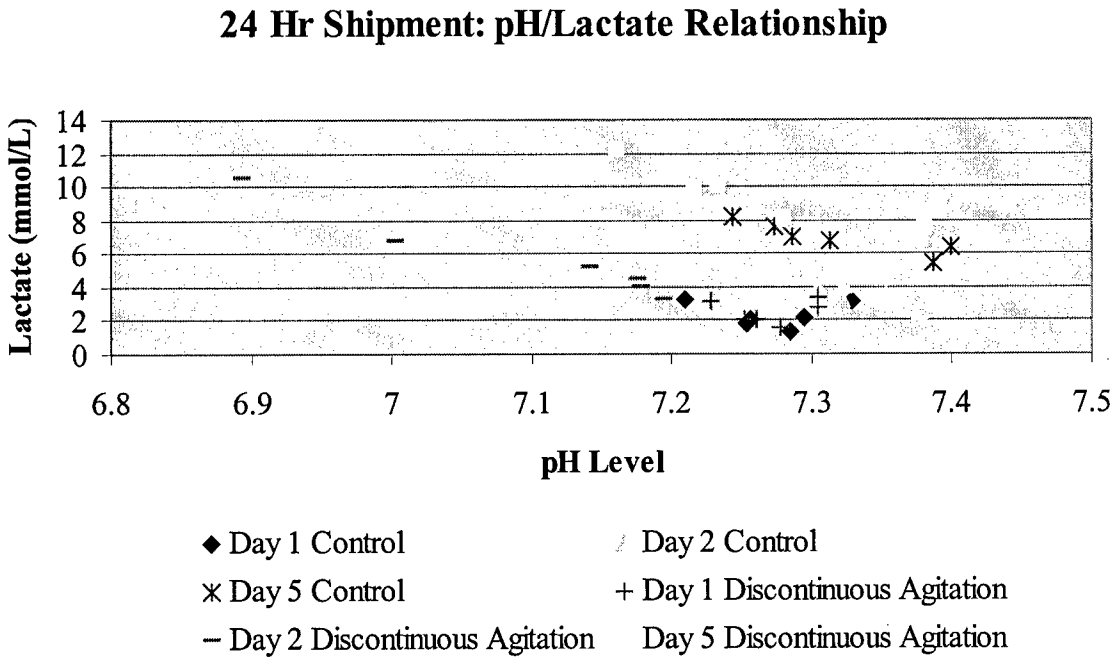

24 Hr Shipment: pH/Lactate Relationship
Lactate (mmol/L)
14
12
10
8
6
4
2
0
6.8
6.9
7
7.1
7.2
7.3
7.4
7.5
pH Level
◆ Day 1 Control
Day 2 Control
✳ Day 5 Control
+ Day 1 Discontinuous Agitation
– Day 2 Discontinuous Agitation
Day 5 Discontinuous Agitation

FIGURE 2.

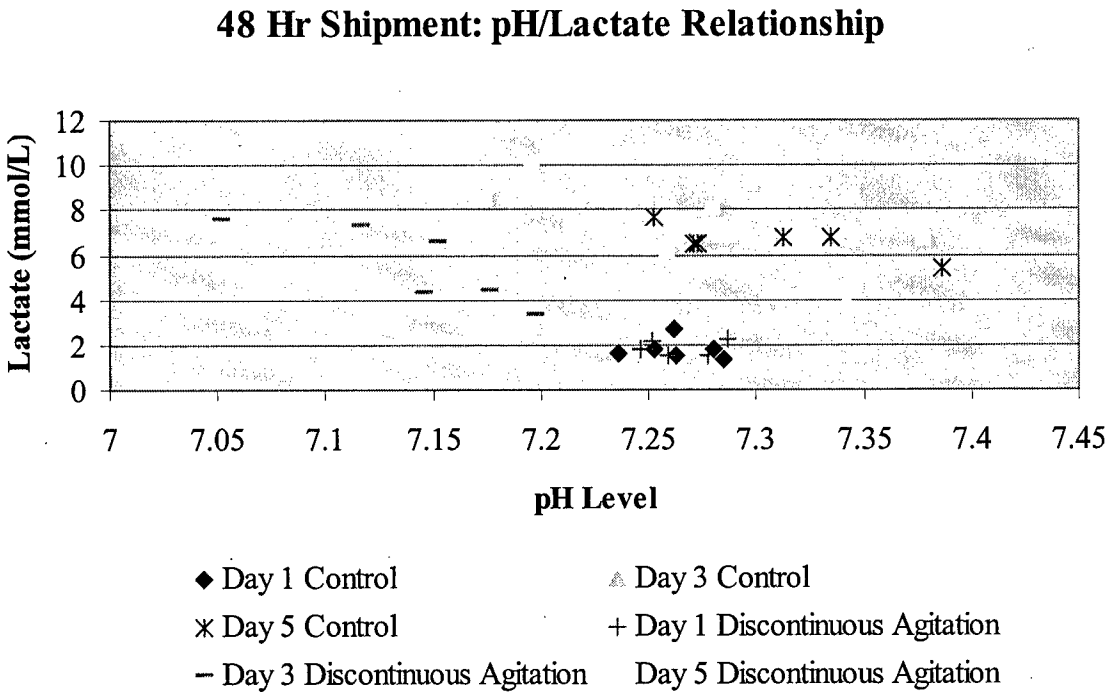
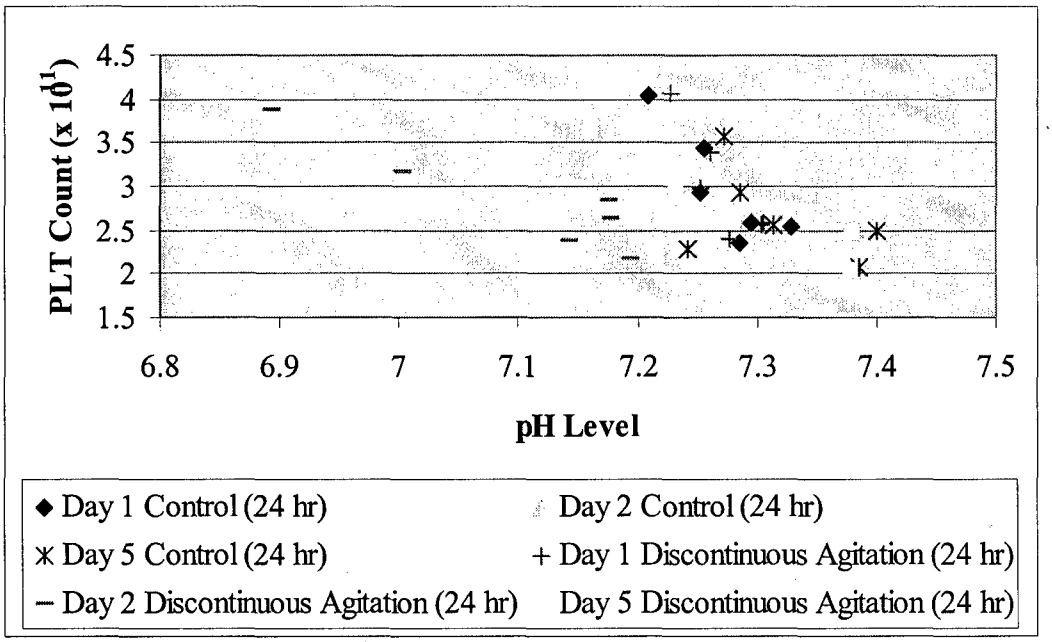

48 Hr Shipment: pH/Lactate Relationship
Lactate (mmol/L)
12
10
8
6
4
2
0
7
7.05
7.1
7.15
7.2
7.25
7.3
7.35
7.4
7.45
pH Level
Day 1 Control
Day 3 Control
Day 5 Control
Day 1 Discontinuous Agitation
Day 3 Discontinuous Agitation
Day 5 Discontinuous Agitation

FIGURE 3.

A


PLT Count (x 10^1)
4.5
4
3.5
3
2.5
2
1.5
6.8
6.9
7
7.1
7.2
7.3
7.4
7.5
pH Level
◆ Day 1 Control (24 hr)
Day 2 Control (24 hr)
✳ Day 5 Control (24 hr)
+ Day 1 Discontinuous Agitation (24 hr)
– Day 2 Discontinuous Agitation (24 hr)
Day 5 Discontinuous Agitation (24 hr)

B

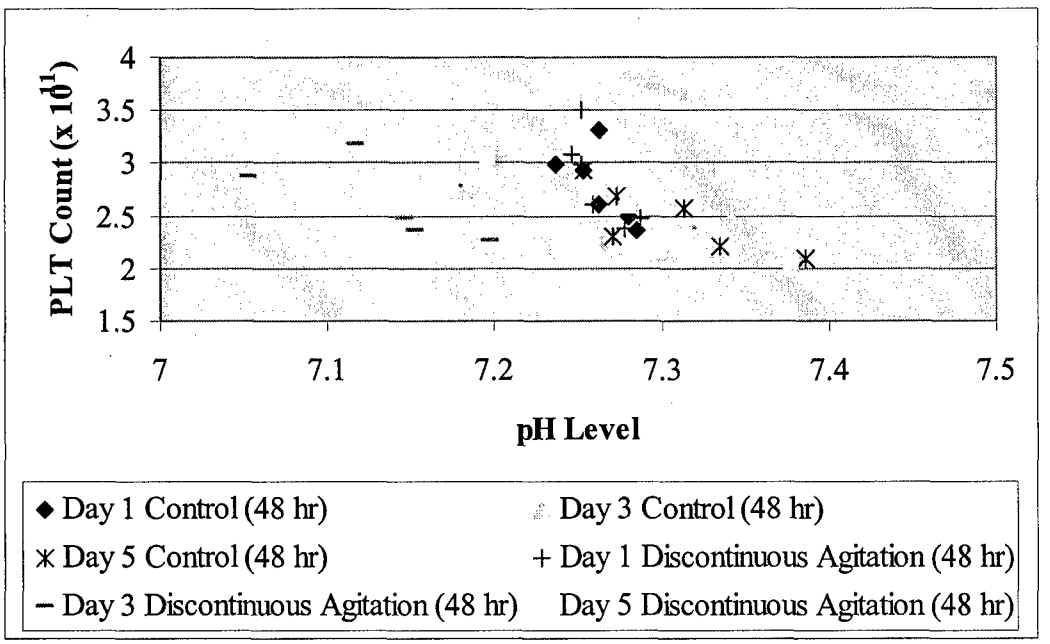
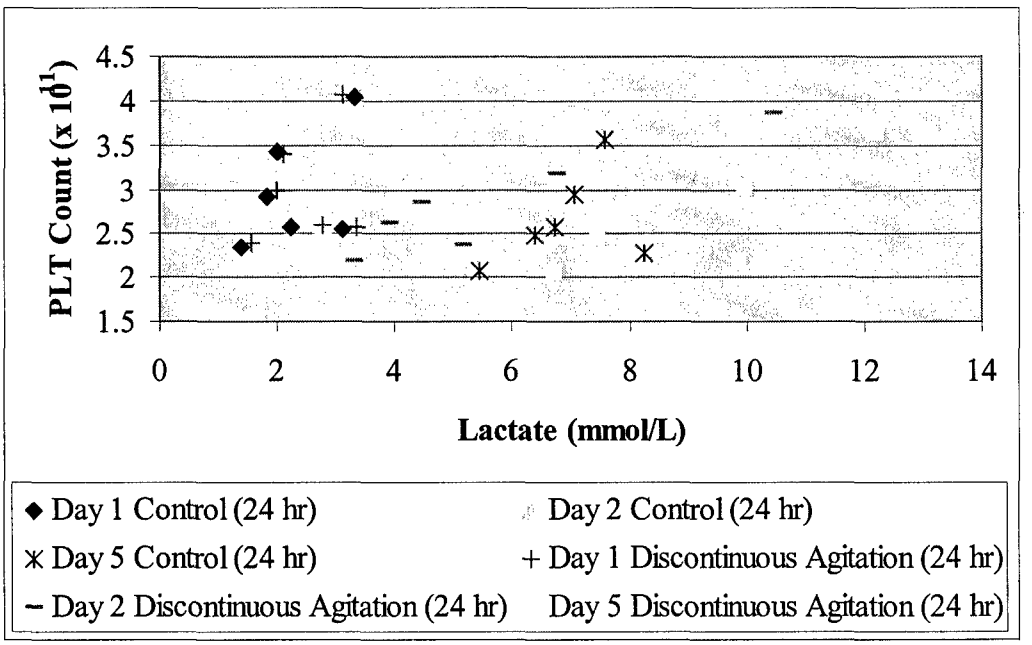

PLT Count (x 10^1)
4
3.5
3
2.5
2
1.5
7
7.1
7.2
7.3
7.4
7.5
pH Level
◆ Day 1 Control (48 hr)
Day 3 Control (48 hr)
✳ Day 5 Control (48 hr)
+ Day 1 Discontinuous Agitation (48 hr)
– Day 3 Discontinuous Agitation (48 hr)
Day 5 Discontinuous Agitation (48 hr)

FIGURE 4.

A


PLT Count (x 10^1)
4.5
4
3.5
3
2.5
2
1.5
0
2
4
6
8
10
12
14
Lactate (mmol/L)
◆ Day 1 Control (24 hr)
Day 2 Control (24 hr)
✳ Day 5 Control (24 hr)
+ Day 1 Discontinuous Agitation (24 hr)
– Day 2 Discontinuous Agitation (24 hr)
Day 5 Discontinuous Agitation (24 hr)

B

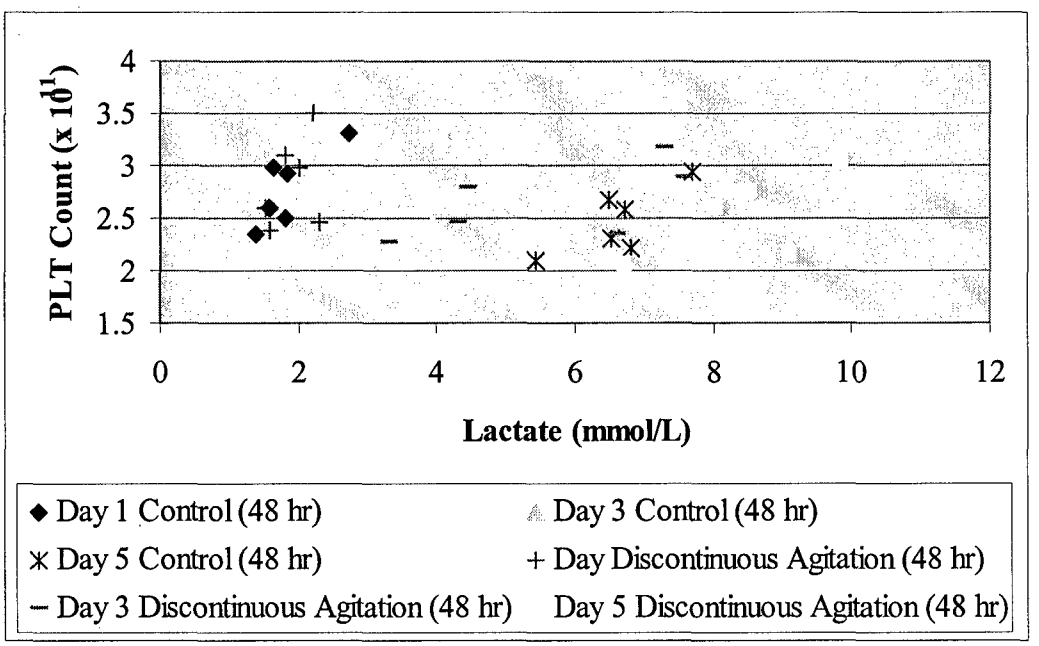
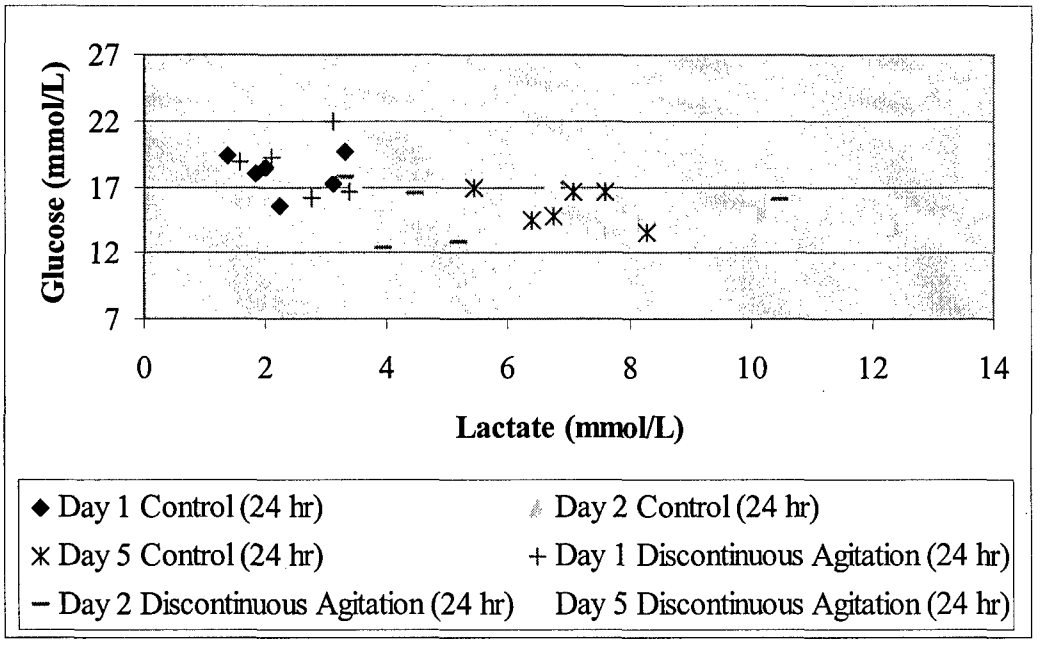

PLT Count (x 10^1)
4
3.5
3
2.5
2
1.5
0
2
4
6
8
10
12
Lactate (mmol/L)
◆ Day 1 Control (48 hr)
Day 3 Control (48 hr)
✳ Day 5 Control (48 hr)
+ Day Discontinuous Agitation (48 hr)
– Day 3 Discontinuous Agitation (48 hr)
Day 5 Discontinuous Agitation (48 hr)

FIGURE 5.

A


Glucose (mmol/L)
27
22
17
12
7
0
2
4
6
8
10
12
14
Lactate (mmol/L)
◆ Day 1 Control (24 hr)
Day 2 Control (24 hr)
✳ Day 5 Control (24 hr)
+ Day 1 Discontinuous Agitation (24 hr)
– Day 2 Discontinuous Agitation (24 hr)
Day 5 Discontinuous Agitation (24 hr)

B

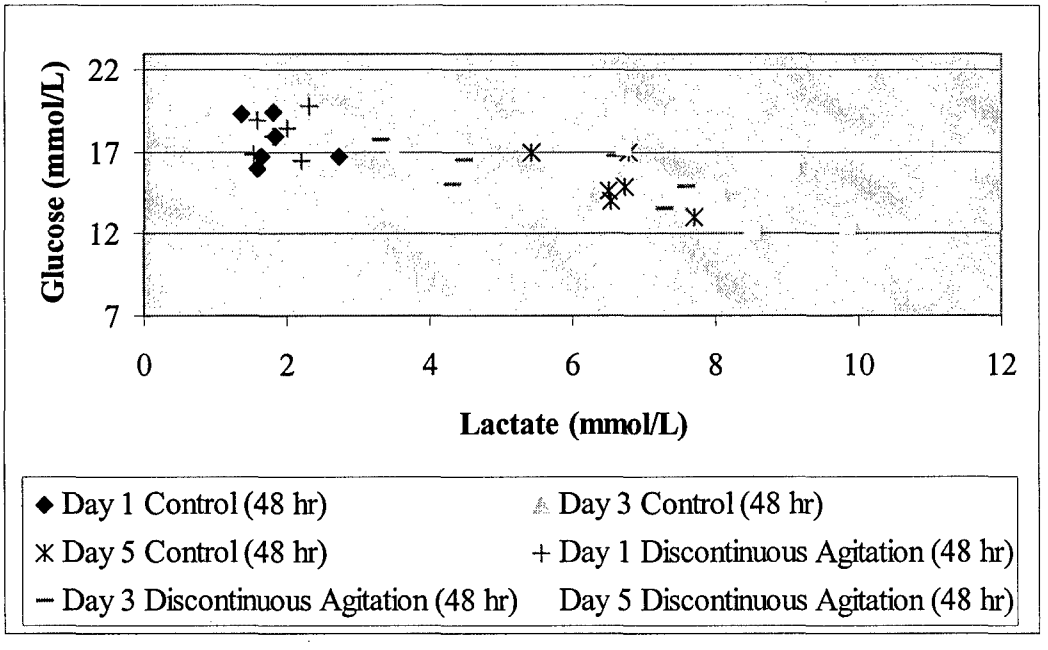
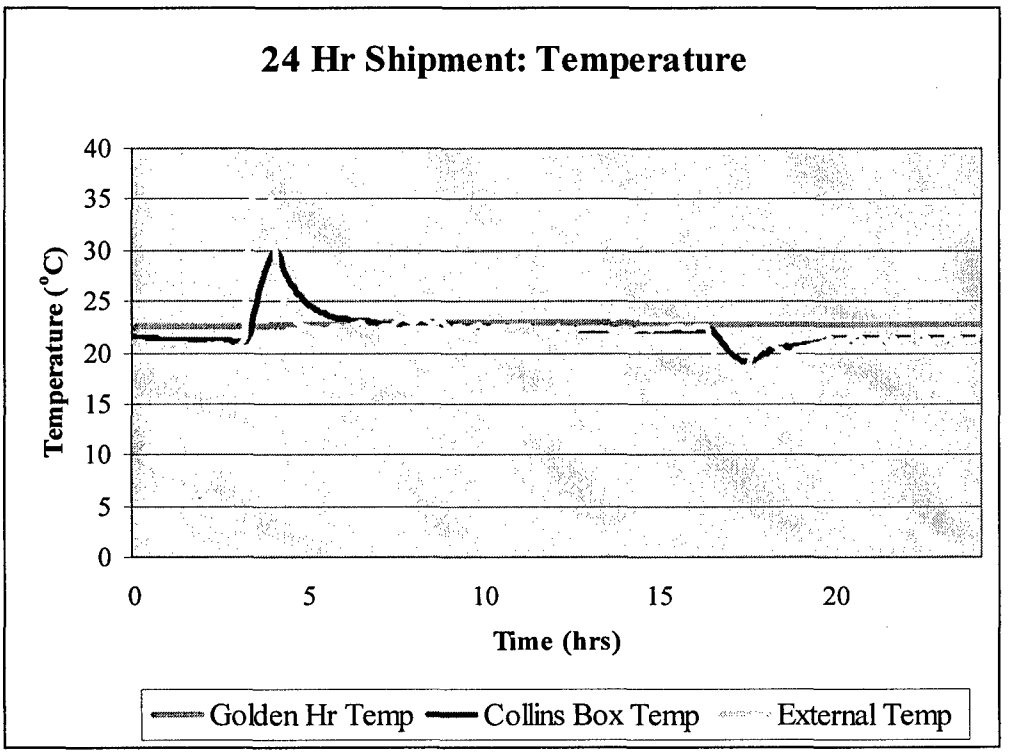
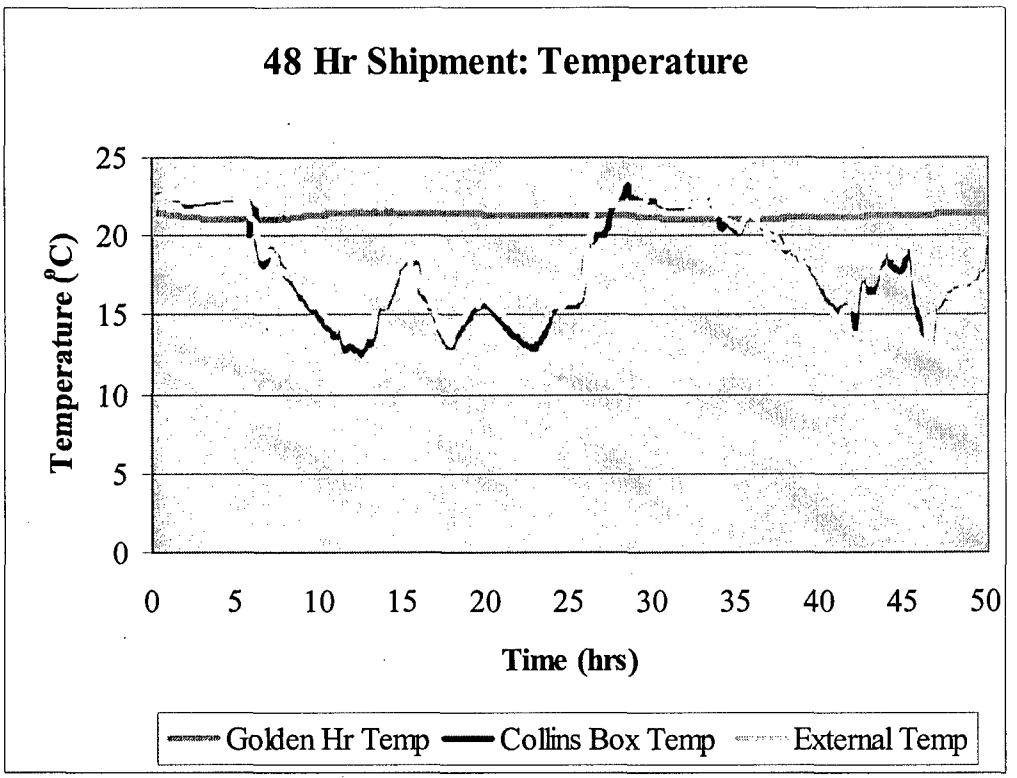

Glucose (mmol/L)
22
17
12
7
0
2
4
6
8
10
12
Lactate (mmol/L)
◆ Day 1 Control (48 hr)
Day 3 Control (48 hr)
✳ Day 5 Control (48 hr)
+ Day 1 Discontinuous Agitation (48 hr)
– Day 3 Discontinuous Agitation (48 hr)
Day 5 Discontinuous Agitation (48 hr)

FIGURE 6.

A


24 Hr Shipment: Temperature
Temperature (°C)
40
35
30
25
20
15
10
5
0
0
5
10
15
20
Time (hrs)
Golden Hr Temp
Collins Box Temp
External Temp

B


48 Hr Shipment: Temperature
Temperature (°C)
25
20
15
10
5
0
0
5
10
15
20
25
30
35
40
45
50
Time (hrs)
Golden Hr Temp
Collins Box Temp
External Temp